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# A COMPOSITION CONTAINING CARVACROL AND THYMOL FOR USE AS A BACTERICIDE

The present invention relates to a composition containing the natural substances carvacrol and thymol. The composition exhibits a synergistic bactericidal effect against Treponema, which causes severe muco-hemorrhagic dieseases, such as swine dysentery and severe diseases affecting the hoofs of hoofed animals, particularly cloven-hoofed animals, such as ruminents. The composition is suitably administered to an animal via a diet composition, drinking water or a drench bath.

Diseases caused by Treponema are common in animal stocks. For example, an anaerobic spirochete, Treponema hyodysenteriae, is considered to be the primary etiologic agent of swine dysentery (SD). See for instance D.L. Harris and R.J. Lysons (1992), Swine dysentery, in: Diseases of swine, 7$^{th}$ edition, Iowa State University Press, Ames, Iowa, USA. SD is a severe muco-hemorrhagic diarrhoea. Pigs actually affected with SD usually consume very low amounts of feed causing an essential reduction in growth and a considerable economical loss. In Top Agrar (1998), Vol. 4, page 52 it is reported that in Germany one third of the piglets herds and as much as one half of the growing pig herds probably are Treponema-positive. Large efforts are therefore justified to prevent the diseases to be spread. It is also well known that Treponema causes severe diseases affecting the hoofs of hoofed animals, particularly cloven-hoofed animals, such as ruminents.

A wide spectrum of antibiotics, such as streptomycin, bacitracin, neomycin, tylosin, gentamycin, chlostetracycline, virginamycin and lincomycin, have been reported to be effective in the treatment of SD. Where the disease is endemic, preventive medication is often added to the animal feed. However, it seems that the antibiotics become less and less effective and Top Agrar (1998), Vol. 4, page 52, reports a resistance ratio of Treponema of 99% to tylosin, 92% to lyncomycin and 48% to tiamulin in 1997.

However, in recent years there has been an intense debate about the use of chemical and antibiotic growth promoters and in many countries a ban on this type of feed additives is being considered. Thus, there is an urgent need for agriculture to develop substances which are in line with reliable and generally accepted practice and not of a medicinal nature.

One objective of the present invention is to provide natural substances as active agents, which are suitable for the administration to the animal via a diet, drinking water or a drench bath for the cure, prevention or alleviation of the diseases caused by Treponema. Another objective is to reduce the negative effects on the growth.

According to the invention it has been found that a composition, containing the natural substances carvacrol in an amount of 5 ppm to 90% by dry weight and thymol in an amount of 5 ppm to 80% by dry weight, in a weight ratio between 1:5 and 10:1, preferably between 2:3 and 4:1, exhibits a synergistic effect against Treponema and thereby diminishing the negative effect these bacteriae have on the health and growth of animals. By the expression "a natural substance" is in this context understood a substance which consists of compounds occurring in nature and that is obtained from natural products or through synthesis. The composition is usually administered as a diet composition or as a drinking water containing carvacrol and thymol in amounts of 5-2000 ppm, preferably 20-600 ppm, calculated on the dry weight of the diet composition including nutritive substances or on the weight of the drinking water. The composition may also be present in an amount of 0.2-30% by weight in a drench bath for the treatment of the hoofs of hoofed animals, particularly cloven-hoofed animals, such as ruminents. The invention also includes a premix and a diet additive that may be used in the preparation of the diet composition as well as a drinking water supplement and a drench bath supplement.

The composition and the diet composition may also contain other natural substances which enhance the health and improve the growth. In the diet composition these substances are normally present in an amount of 0.1 to 30 ppm, calculated on the dry weight. Examples of such substances and their amounts are 1-5 ppm guaiacol, 1-5 ppm eugenol, 0.1-2 ppm capsascin and 1-20 mg tannin. Other suitable ingredients in the diet composition are flavourings of natural substances. They are usually present in an amount of 0.2-50 ppm, calculated on the dry weight of the diet composition. Examples of suitable flavourings and their amounts are 0.05-0.5 ppm creosol, 0.1-5 mg anethole, 0.1-2 ppm of deca-, undeca- and/or dodecalactones, 0.1-2 quinoleine, 0.1-2 ppm ionones and/or irone, 0.05-1 ppm gingerol, 0.05-2 ppm piperine, 0.05-1 ppm propylidene and/or butylidene phtalides and 0.1-5 ppm amyl and/or benzyl salicylate.

The incorporation of active ingredients into the diet composition is usually carried out by preparing a premix of the active compounds carvacrol and thymol and other suitable additives. Such a premix may contain 1-10% by dry weight of carvacrol and thymol, 0-40% by dry weight of growth improving additives, flavourings and health enhancing additives, and 50-99% by weight of an absorbing support. The support may contain, for example, 40-50% by weight of wood fibres, 8-10% by weight of stearin, 4-5% by weight of curcuma powder, 4-5% by weight of rosemary powder, 22-28% by weight of limestone, 1-3% by weight of a gum, such as gum arabic, 5-50% by weight of sugar and/or starch and 5-15% by weight of water.

This premix can then be mixed with common feed components, such as vitamins, enzymes, mineral salts, ground cereals, protein-containing components, carbohydrate-containing components, wheat middlings and/or brans in the preparation of a diet composition additive which contains 0.2-5% by weight of the premix. The diet composition additive is then finally added to the diet composition in such quantities that the feed will contain 5-2000 ppm,

preferably 20-600 ppm, of the active mixture. The diet composition additive normally constitutes 0.3-3.5% by weight of diet composition.

The diet composition according to the invention usually contains, calculated on the dry weight of the feed, the following ingredients:

a) 0-80%, preferably 10-70%, by weight of cereals,

b) 0-30%, preferably 1-12%, by weight of fat,

c) 0-85%, preferably 10-50%, by weight of protein containing nutritious substances of a type other than cereals, and

d) 1-500 ppm, preferably 10-100 ppm, of the mixture.

The total amounts of a)-d) are preferably at least 80% by weight.

When preparing the diet composition, the diet composition additive can be mixed with the dry ingredients consisting of cereals, such as ground or crushed wheat, oats, barley, maize and rice; vegetable protein feed based on e.g. rapeseed, soya bean and sunflower; animal protein feed, such as blood meal, meat and bone meal and fish meal; molasses; and milk products, such as various milk powders and whey powders. After mixing all the dry additives, the liquid ingredients and ingredients, which after heating become liquid, can be added. The liquid ingredients may consist of lipids, such as fat, for example slaughter fat and vegetable fat, optionally liquefied by heating, and/or of carboxylic acids, such as a fatty acid. After thorough mixing, a mealy or particulate consistency is obtained, depending on the degree of grinding of the ingredients. To prevent separation during storage, water should preferably be added to the animal feed, which then is subjected to a conventional pelletising, expanding or extruding process. Any excess water can be removed by drying. If desired, the resulting granular animal feed can also be crushed to a smaller particle size.

The drinking water supplement may contain 2-90% by dry weight, preferably 10-50% by dry weight, of carvacrol and thymol. Beside carvacrol and thymol the supplement also

contains 10-98% by dry weight of a large number of other ingredients. Common ingredients are mineral salts, vitamines, natural substances enhancing the health and growth, flavourings, water-soluble or water-dispersable carriers, such as sugars, powdered milk, milk-by-products and cellulose derivatives, dispersing agents and stabilisers, such as water-soluble or water-dispersable polymers. Suitable examples of natural substances enhancing the health and growth have earlier been described. When preparing the drinking water, the supplement is normally added to the water in such an amount that the concentration of the natural substance becomes 5-2000 ppm, preferably 20-600 ppm.

The drench bath supplement may contain 30-98% by dry weight of carvacrol and thymol and 2-70% by weight of mineral salts, water-soluble or water-dispersable carriers, dispersing agents and/or stabilisers, such as water-soluble or water-dispersable polymers.

Within the scope of the invention, it is also possible to produce a suspension of the diet composition. This is especially convenient if the feed is prepared for immediate consumption.

The present invention will now be further illustrated by the following Examples.

**Example 1**

The antimicrobial activity of the composition of the invention towards Treponema innocens and Triponema hyodysenteriae was determined in vitro. In the tests the following organisms, growth media, culture conditions and evaluation method were used.

| | |
|---|---|
| Organisms: | Treponema innocens, ATTC 29796<br>Treponema hyodysenteriae, ATCC 31212 |
| Growth media: | Caseine-peptone soymeal-peptone agar USP (Caso-Agar, Merch No. 5458) + 5% Sheep blood |

Culture conditions: Anaerobic incubation at 37°C for 4-6 days

Evaluation method: Agar dilution test (according to DIN 58940, teil 6)

Agar plates were prepared by using the growth media, to which 10% by weight of a solution of carvacrol and/or thymol in polypropylene glycol had been added.

Cell suspensions with a concentration of 10.9 cfu/ml were prepared of each of the organisms. The single suspensions were then distributed on the agar surface using a Multipoint inoculator applying 1µl to a final surface of about 0.5 $cm^2$. For every concentration of carvacrol and/or thymol two parallel plates were inoculated and on each plate three inoculation points of each of the two organisms are applied. After the inoculation period the growth of the organisms were observed. If no growth was observed, the concentration of carvacrol and/or thymol was in the next test reduced to half. The minimum concentration of carvacrol/and or thymol leading to a total suppression of bacterial growth is noted as the MIC value (minimal inhibitory concentration) of the active components or compounds. The following results were obtained.

| Test No. | Active compound | MIC value | |
|---|---|---|---|
| | | Treponema innocens | Treponema hyodysenteriae |
| 1 | Thymol | 625 | 625 |
| 2 | Carvacrol | 313 | 313 |
| 3 | 2/3 Thymol 1/3 Carvacrol | 156 | 156 |
| 4 | 1/2 Thymol 1/2 Carvacrol | 156 | 156 |
| 5 | 1/3 Thymol 2/3 Carvacrol | < 78 | < 78 |

From the results it is evident that the composition of the invention exhibits a synergistic antimicrobial effect on the tested organisms.

**CLAIMS**

1. A composition **characteriz d in,** that it contains the natural substances carvacrol in an amount of 5 ppm to 90% by dry weight, and thymol in an amount of 5 ppm to 80% by dry weight, the weight ratio between carvacrol and thymol being from 1:5 to 10:1.

2. A composition **characterized in**, that the weight ratio is from 2:3 to 4:1.

3. A diet composition according to claims 1 or 2, **characterized in**, that it contains carvacrol and thymol in amounts of 5-2000 ppm, preferably 20-600 ppm calculated on dry weight of the diet composition.

4. A diet composition according to claim 3, **characterized in**, that the diet composition contains 0.1-30 ppm calculated on the dry weight of natural substances, selected from the group consisting of guaiacol, eugenol, capsascin and tannin, which enhance the health and increase the growth, and/or 0.2-50 ppm calculated on the dry weight of natural substances, selected from the group consisting of creosol, anethole, deca-, undeca- and/or dodecalactones, quinoleine, ionones and/or irone, gingerol, piperine, propylidene and/or butylidene phtalides, amyl and/or benzyl salicylate.

5. A diet composition according to claim 4, **characterized in**, that it contains

a) 10-70% by weight of cereals,

b) 1-12% by weight of feed fat, and

c) 10-50% by weight of protein containing nutritious substances of a type other than cereals.

6. A premix according to claim 1 or 2, **characterized in**, that it contains 1-10% by dry weight of carvacrol and thymol, 0-40% by dry weight of growth improving additives, health enhancing additives and flavourings and 50-99% by dry weight of an absorbing support.

7. A diet additive, **characterized in**, that it contains 0.2-5% by dry weight of the premix in claim 6, 80-99% by dry weight of vitamins, enzymes, mineral salts, ground cereals,

protein components, carbohydrate components, wheat middlings and/or bran.

8. A drinking water supplement according to claims 1 or 2, **characterized in**, that it contains 2-90% by dry weight of carvacrol and thymol.

9. A drench bath supplement according to claims 1 or 2, **characterized in**, that it contains 30-98% by dry weight of carvacrol and thymol.

10. Use of a composition according to claim 1 or 2 as a bactericide.

11. A composition according to any one of claims 1-8 for use as a medicament.

12. A composition according to claim 11 where the medicament is used against swine dysentery.

13. A composition according to claim 11, where the medicament is used in a drench bath for the treatment of the hoofs of hoofed animals.

**ABSTRACT**

The present invention relates to a composition containing the natural substances carvacrol and thymol. The amount of carvacrol is 5 ppm to 90% by dry weight, the amount of thymol is 5 ppm to 80% by dry weight, and the weight ratio between carvacrol and thymol is from 1:5 to 10:1.

The composition exhibits a synergistic bactericidal effect against Treponema which causes severe muco-hemorrhagic diseases, such as swine dysentery and severe diseases affecting the hoofs of hoofed animals, particularly cloven-hoofed animals, such as ruminents. The composition is suitably administered to an animal via the diet or via the drinking water or via a drench bath.